

AMENDMENT AND RESPONSE UNDER 37 CFR § 1.111  
Serial Number: 09/643,128  
Filing Date: August 21, 2000  
Title: Gene Inactivation by targeted DNA methylation

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Dkt: GMR-001

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**Amendments to the Specification:**

Please amend the specification as follows:

In the paragraph beginning on line 1 of page 11, the following changes are made:

In one embodiment, the first strand includes two m5CG sequences, and the second strand includes two corresponding unmethylated CG sequences, thus forming a duplex having two hemi-methylated sites. For example, the first strand can include a 5'-m5CGN4m5CG-3' (SEQ ID NO:aa) sequence, where N4 is any nucleotide. In one variation of this embodiment, the first strand can include the sequence 5'-mCGTm5CG-3' (SEQ ID NO:bb). In another embodiment, the first strand includes two m5CN1G sequences, and the second strand includes two corresponding unmethylated CN2G sequences, also forming a duplex having two hemi-methylated sites. Alternatively, the first strand can include an m5CG sequence and an m5CN1g sequence, with the second strand including an unmethylated CG sequence and an unmethylated CN2G sequence.

In the paragraph beginning on line 3 of page 12, the following changes are made:

In one embodiment, the linker joining the first and second strands of the imprinting element is a single nucleotide, for example, a single thymine. A preferred imprinting element of this type has the sequence of 5'-CGACG-T-m5CGTm5CG-3' (SEQ ID NO: [[cc]] 1; with the linker thymine shown in bold).

In the paragraph beginning on line 26 of page 13, the following changes are made:

In an exemplary embodiment, the guiding element is a 22-nucleotide oligomer having the sequence 5'-AGCCm5CGGGm5CTGGGAGGAGTm5C GG-3' (SEQ ID NO: [[dd]] 2), which targets an Igf2 promoter. A preferred polynucleotide having a preferred imprinting element linked to this guiding element has the sequence: 5'-CGACGTm5CGTm5CGAGCCm5CGGGm5CTGGGAGGAGTm5CGG-3' (SEQ ID NO: [[zz]] 3). This polynucleotide directs methylation at a target nucleotide sequence in the Igf2 promoter.

Starting at line 10 of page 14, the table is changed as shown below:

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c-myc	T <u>C</u> G CTA ATC T <u>C</u> C GCC CAC <u>C</u> GG (SEQ ID NO: 4) ACC GGC CCT TTA TAA T <u>G</u> C GA (SEQ ID NO: 5) T <u>C</u> C GCC CAC <u>C</u> GG CCC TTT AT (SEQ ID NO: 6)
HIV promoter	CAC GTA GCC <u>C</u> GA GAG <u>C</u> TG (SEQ ID NO: 7) CCC GAG AG <u>C</u> TGC ATC <u>C</u> GG (SEQ ID NO: 8) G <u>C</u> T GCA TAT AAG <u>C</u> AG <u>C</u> TG (SEQ ID NO: 9)
human urokinase plasminogen activator receptor (uPAR)	AGG <u>C</u> GC CCA <u>C</u> GC ATC TGG (SEQ ID NO: 10) T <u>C</u> G CTC TTT <u>C</u> GC AAA A <u>C</u> G T (SEQ ID NO: 11) A <u>C</u> G CAT <u>C</u> TG GGG <u>C</u> TG ACT (SEQ ID NO: 12)
human vascular endothelial growth factor receptor (flt-1)	GTT ATA AAT <u>C</u> GC CCC <u>C</u> GC (SEQ ID NO: 13) G <u>C</u> T GGG GAA AGG TTA TAA AT <u>C</u> GC (SEQ ID NO: 14) ACC CCT TGA <u>C</u> GT CAC <u>C</u> AG (SEQ ID NO: 15) CTT CAT <u>C</u> GA GGT <u>C</u> CG <u>C</u> GG (SEQ ID NO: 16)
human vascular endothelial growth factor receptor (KDR/FLK-1)	C <u>C</u> T GCA <u>C</u> TG AGT CCC <u>C</u> GG (SEQ ID NO: 17) A <u>C</u> G GGA GAG CCC CTC CTC <u>C</u> GC (SEQ ID NO: 18)
human $\beta$ 3 integrin gene	CAC TGT GGG G <u>C</u> G G <u>C</u> C GGA (SEQ ID NO: 19) T <u>G</u> C GTC CCA CCC ACC G <u>C</u> G (SEQ ID NO: 20)
human 12-lipoxygenase	<u>C</u> CG CAG ACC GGT CCT TTA A (SEQ ID NO: 21) C <u>C</u> T GGG <u>C</u> GG TCC <u>C</u> GG GCA (SEQ ID NO: 22)
human $\beta$ -amyloid protein precursor ( $\beta$ -APP)	CTC <u>C</u> GT <u>C</u> AG TTT CCT <u>C</u> GG C (SEQ ID NO: 23) AT <u>C</u> AG <u>C</u> TGA CTC G <u>C</u> C TGG (SEQ ID NO: 24)
human vascular	TAG <u>C</u> GG GGA GGA T <u>C</u> G <u>C</u> GG A (SEQ ID NO: 25)

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epithelial growth factor (VEGF)	TAA AAG TCG GCT GGT AGC GG (SEQ ID NO: 26)
human insulin like growth factor-1 (IGF1)	TCT GTG CTC TAG TTT TAA (SEQ ID NO: 27)
	CCA GCT GTT TTC CTG TCT (SEQ ID NO: 28)
human epidermal growth factor receptor (HER2)	GCT GCT TGA GGA AGT ATA AG (SEQ ID NO: 29)
	AGA ATG AAG TTG TGA AGC T (SEQ ID NO: 30)
human tumor necrosis factor $\alpha$ (TNF- $\alpha$ )	TGC CGT TCC TCT ATA AAG (SEQ ID NO: 31)
	AGG GAC CTG AGC GTC CGG (SEQ ID NO: 32)
human tumor necrosis factor $\beta$ (TNF- $\beta$ )	TCG CCC CAG GGA CAT ATA AAG (SEQ ID NO: 33)
	CAT ATA AAG GCA GTT GTT (SEQ ID NO: 34)
	ACC CAG CCA GCA GAC GCT (SEQ ID NO: 35)
human interleukin 4 (IL-4)	TCG GTT TCA GCA ATT TTA (SEQ ID NO: 36)
	TAG AGA TAT CTT TGT CAG C (SEQ ID NO: 37)
human granulocyte-macrophage colony stimulating factor (GM-CSF)	CTC TGT GTA TTT AAG AGC T (SEQ ID NO: 38)
	CCG CCT CCC TGG CAT TTT G (SEQ ID NO: 39)
human interleukin 2 (IL-2)	CCA GAG AGA AGA GTA TAA T (SEQ ID NO: 40)
human bcl-2	ATA GCT GGA TTA TAA CTC (SEQ ID NO: 41)
	TCG TCC AAG AAT GCA AAG (SEQ ID NO: 42)
hepatitis B virus (HBV)	CAG CCA TGG AAA CGA TGT (SEQ ID NO: 43)
	TGA AGC GAA GTG CAC ACG G (SEQ ID NO: 44)
	AGA CGG TGA GAC CGC GTA (SEQ ID NO: 45)

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	TGC ATG GTG CTG GTG <u>C</u> GC A (SEQ ID NO: <u>46</u> )
Cytomegalovirus	TGG <u>G</u> CG GTA GGC <u>G</u> TG TAC <u>G</u> G (SEQ ID NO: <u>47</u> )
(CMV)	<u>A</u> CG GTA AAT GGC <u>C</u> CG <u>C</u> CT G (SEQ ID NO: <u>48</u> )
	<u>G</u> CG TCA ATG GGG <u>C</u> GG AGT (SEQ ID NO: <u>49</u> )
human c-fos	<u>A</u> CG CTT GTT ATA AAA <u>G</u> CA GT (SEQ ID NO: <u>50</u> )
	<u>T</u> CG TAC TCC AAC <u>C</u> GC ATC <u>T</u> G (SEQ ID NO: <u>51</u> )
human raf-1	<u>C</u> CG AGA GTC TTA ATC <u>C</u> CG G (SEQ ID NO: <u>52</u> )
	<u>T</u> CG <u>C</u> GC <u>A</u> GA ATC <u>G</u> GA GGC (SEQ ID NO: <u>53</u> )

In the paragraph beginning at line 19, page 25, please make the following changes:

An oligonucleotide having the sequence: 5' CGACGTm5CGTm5CGAGCCm5CGGGm5CTGGGAGGAGTm5CGG-3' ("HepKex;" SEQ ID NO: [[zz]] 3) was designed to target the most proximal promoter of Igf2 (human hP4 and mouse mP3). This oligonucleotide and a control oligonucleotide ("CTII001") having the sequence: 5'-GGTCACGGTCAGGCGTAGATGG-3' (SEQ ID NO: [[xx]] 54) were synthesized as phosphorothionate deoxyoligonucleotides using standard automated phosphoramidite chemistry and were purified by HPLC. (The CTII001 control oligonucleotide had the same nucleotide content as HepKex, but with a randomized sequence.)

In the paragraph beginning at line 27 on page 25, make the following changes:

More specifically, HepKex was synthesized using phosphorothionate deoxynucleotide precursors, except that a methylated cytidine precursor (5mdC) was used to introduce methylated cytidines at desired positions in the oligonucleotide, HepKex was synthesized as a single stranded oligonucleotide and, after HPLC, was dissolved in aqueous solution. In this solution, the imprinting element portion of HepKex

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(residues 1-11 of SEQ ID NO: 3) (~~5'-CGACG-T-m<sup>5</sup>CGT-m<sup>5</sup>CG-3~~ SEQ ID NO: ??) self  
anneals to form a hairpin structure.

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